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Health and Environmental Effects Profile for Phenylenediamines

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16. ABSTRACT

The Health and Environmental Effects Profile for phenylenediamines was prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA) and to provide health-related limits for emergency actions under Section 101 of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained from Agency program office files were evaluated as they pertained to potential human health, aquatic life and environmental effects of hazardous waste constituents. Quantitative estimates have been presented provided sufficient data are available. Existing data are insufficient to determine an Acceptable Daily Intake (ADI) or a carcinogenic potency factor for the class as a whole. An ADI for p-phenylenediamine of 13 mg/day has been calculated, and a q_1^* of 4.68×10^{-2} $(mg/kg/day)^{-1}$ has also been calculated. The Reportable Quantity (RQ) value of 1, 10, 100, 1000 or 5000 pounds is used to determine the quantity of a hazardous substance for which notification is required in the event of a relase as specified by CERCLA based on chronic toxicity. Existing data are insufficient to determine an RQ value for the class as a whole. An RQ of 5000 for p-phenylenediamine has been derived.

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HEALTH AND ENVIRONMENTAL EFFECTS PROFILE FOR PHENYLENEDIAMINES

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PREFACE

Health and Environmental Effects Profiles (HEEPs) are prepared for the Office of Solid Waste and Emergency Response by the Office of Health and Environmental Assessment. The HEEPs are intended to support listings of hazardous constituents of a wide range of waste streams under Section 3001 of the Resource Conservation and Recovery Act (RCRA), as well as to provide health-related limits for emergency actions under Section 101 of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA). Both published literature and information obtained from Agency program office files are evaluated as they pertain to potential human health, aquatic life and environmental effects of hazardous waste constituents. The literature searched and the dates of the searches are included in the section titled "Appendix: Literature Searched." The literature search material is current through November, 1984.

Quantitative estimates are presented provided sufficient data are available. For systemic toxicants, these include Acceptable Daily Intakes (ADIs) for chronic exposures. An ADI is defined as the amount of a chemical to which humans can be exposed on a daily basis over an extended period of time (usually a lifetime) without suffering a deleterious effect. In the case of suspected carcinogens, ADIs are not estimated in this document series. Instead, a carcinogenic potency factor of q_1^* (U.S. EPA, 1980) is provided. These potency estimates are derived for both oral and inhalation exposures where possible. In addition, unit risk estimates for air and drinking water are presented based on inhalation and oral data, respectively.

Reportable quantities (RQs) based on both chronic toxicity and carcinogenicity are derived. The RQ is used to determine the quantity of a hazardous substance for which notification is required in the event of a release as specified under CERCLA. These two RQs (chronic toxicity and carcinogenicity) represent two of six scores developed (the remaining four reflect ignitability, reactivity, aquatic toxicity and acute mammalian toxicity).

The first draft of this document was prepared by Syracuse Research Corporation under EPA Contract No. 68-03-3228. The document was subsequently revised after reviews by staff within the Office of Health and Environmental Assessment: Carcinogen Assessment Group, Reproductive Effects Assessment Group, Exposure Assessment Group, and the Environmental Criteria and Assessment Office in Cincinnati.

The HEEPs will become part of the EPA RCRA and CERCLA dockets.

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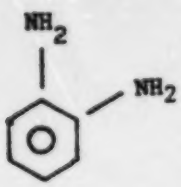
LIST OF ABBREVIATIONS

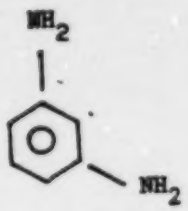
ADI	Acceptable daily intake
CS	Composite score
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
LD_{50}	Dose lethal to 50% of recipients
MED	Minimum effective dose
MTD	Maximum tolerated dose
NOAEL	No-observed-adverse-effect level
ppm	Parts per million
RQ	Reportable quantity
RV_d	Dose-rating value
RV_e	Effect-rating value
STEL	Short-term exposure level
TLC	Thin-layer chromatography
TLV	Threshold limit value
TWA	Time-weighted average

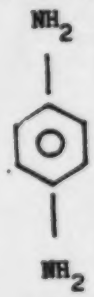
1. INTRODUCTION

1.1. STRUCTURE AND CAS NUMBER

Phenylenediamine is the generic name for a large number of substituted diaminobenzenes. Unless specifically stated, the name, phenylenediamine, when used in this document, refers only to the unsubstituted three isomeric phenylenediamines, o-diaminobenzenes (1,2-benzenediamine), m-diaminobenzene (1,3-benzenediamine) and p-diaminobenzene (1,4-benzenediamine). The structural and empirical formulas for the three phenylenediamines are as follows:







Name:	o-phenylenediamine	m-phenylenediamine	p-phenylenediamine
Empirical formula:	$C_6H_8N_2$	$C_6H_8N_2$	$C_6H_8N_2$
Molecular Weight:	108.15	108.15	108.15
CAS Registry number:	95-54-5	108-45-2	106-50-3

1.2. PHYSICAL AND CHEMICAL PROPERTIES

All three isomers are white solids, which darken on exposure to air (Layer, 1978). The physical properties of the phenylenediamines are presented in Table 1-1.

TABLE 1-1

Physical Properties of Phenylenediamines

	o-Phenylenediamine	m-Phenylenediamine	p-Phenylenediamine	Reference
Melting point (°C):	102	63	140	Layer, 1978
Boiling point (°C):	256-258 (decomposition)	287	267	Layer, 1978
Water solubility (mg/ℓ)	41,500 (at 35°C)	351,000 (at 25°C)	38,000 (at 24°C)	Verschueren, 1983
Log octanol/water partition coefficient	0.15	0	-0.25	Federal Register, 1982
pk_b	9.49	9.12	7.96	Layer, 1978
Ionization potential (eV)	7.29	7.26	6.52	Layer, 1978

1.3. PRODUCTION DATA

No data on production volume for the three phenylenediamines were found in the available literature. In 1974, however, production volume of substituted p-phenylenediamines amounted to 33,550 metric tons (Layer, 1978). Production locations in the United States for these compounds as of January, 1984 are presented in Table 1-2.

1.4. USE DATA

Phenylenediamines are used in a wide variety of applications. o-Phenylenediamine is not used extensively, although it is used as an intermediate in fungicide and dye synthesis, and as an identification agent in the analysis of 1,2-diketones, carboxylic acids and aldehydes (Federal Register, 1982; Layer, 1978).

m-Phenylenediamine is used in the synthesis of azo dyes, as a hair dye, chemical intermediate, photographic developing agent, reagent, antioxidant/ozonant intermediate, fiber production (Nomex), and a vulcanization accelerator (Layer, 1978; Federal Register, 1982).

p-Phenylenediamine is used as a dye intermediate and as a dye, as well as a chemical intermediate and a photographic developing agent (Federal Register, 1982). The p-isomer is also a monomer in the production of Kevlar and Kevlar-49 polymers, as well as certain fully aromatic polyamids (aramids) (Layer, 1978) and in the production of antiozonites used in rubber tires (Hollifield and Lombardo, 1975). Annual production of Kevlar is >4.5 metric tons (Layer, 1978).

TABLE 1-2

Phenylenediamine Production Facilities in the United States as of January, 1984*

Facility	o-Phenylenediamine	m-Phenylenediamine	p-Phenylenediamine
E.I. du Pont de Nemours and Co., Inc. Deepwater, NJ	X	X	X
Southland Corp. Great Meadows, NJ	-	X	-
Ciba-Geigy Corp. Toms River, NJ	X	-	-
The B.F. Goodrich Co. Henry, IL	-	-	X

*Source: SRI International, 1984

X = Production

- = No production

2. ENVIRONMENTAL FATE AND TRANSPORT PROCESSES

2.1. AIR

No data were found in the available literature as cited in Appendix A detailing the fate of phenylenediamines in air. One study (Nishi and Nishioka, 1982) reports the photolysis of the p-isomer in DMSO at 20°C. The light source used was a Toshiba fluorescent lamp (15 watts). The authors reported that after exposure the solution turned reddish brown and then dark brown. TLC analysis of the mixture yielded a deep purple product (on the silica gel) which the authors suggested was Bandrowski's base (Figure 2-1). This base is also the product of peroxide oxidation of p-phenylenediamine (Layer, 1978). Since the phenylenediamines darken on exposure to air, it is likely that these compounds will undergo autooxidation or oxidation with oxygen in air.

2.2. WATER

Based on the study by Nishi and Nishioka (1982), phenylenediamines will probably photolyze in water. Biodegradation probably will be slow, although the data were somewhat conflicting. Autooxidation will also occur, as will peroxy radical oxidation.

A number of authors have reported on the biodegradability of phenylenediamines; the majority of reports used sewage sludge as an inoculum. Pitter (1976) reported that the o- and m-isomers were "biologically hard to decompose," while the p-isomer was "comparatively well degradable" using an adapted activated sludge. Marion and Malaney (1964), Malaney (1960), Chambers et al. (1963) and Alexander and Lustigman (1966) all reported that the isomeric phenylenediamines are resistant to biodegradation. The inocula used by these investigators were activated sludge from municipal sewage treatment plants, aniline-acclimated activated sludge, phenol-adapted

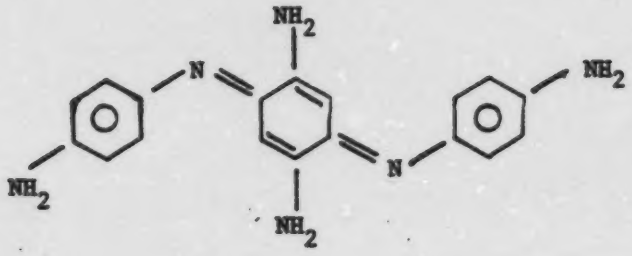


FIGURE 2-1

Bandrowski's Base

activated sludge and soil. The times allowed for degradation to occur were between 0.5 hours and 64 days. Horitsu et al. (1977) reported that Bacillus subtilis readily degraded the p-isomer over an unspecified period.

Corbett (1972) reported that basic (pH 8.5-11) aqueous solutions of p-phenylenediamine autooxidized to Bandrowski's base. The oxidation first involved the formation of p-quinone diamine, which later condensed to form Bandrowski's base.

Young et al. (1973) reported that N-methyl-substituted phenylenediamines quench singlet molecular oxygen by the formation of a charge-transfer-like complex in methanol. This rapid reaction may also be occurring in the photooxidation of phenylenediamine in water, although no direct evidence for it exists. Likewise, a one-electron oxidation of phenylenediamines by hydroxyl radical may occur in water (Rao and Hayon, 1975), although its importance under environmental conditions is not known.

Mill (1980), based on the reaction of alkyl peroxy radicals ($RO_2\cdot$) in solvents, predicted a half-life of ~1 day for aromatic amines (phenylenediamines are aromatic amines) in water. This prediction is based on an average $RO_2\cdot$ reaction rate constant of $1x10^4 M^{-1}S^{-1}$ for aromatic amines and a concentration of $1x10^{-9}$ M for $RO_2\cdot$ radicals in environmental waters.

No information was found in the available literature concerning sorption to sediments; however, given the very low log K_{ow} values and the water solubilities for the phenylenediamines, little or no sorption will take place.

2.3. SOIL

No information concerning the fate of phenylenediamines in soil was found in the available literature as cited in Appendix A. Given the solubility and low log K_{ow} values for these compounds, considerable leaching may occur. The investigation of Alexander and Lustigman (1966) indicates that biodegradation in soil may be a slow process.

2.4. SUMMARY

Based on the available information specific to phenylenediamines and some generalizations concerning aromatic amines, the dominant fate processes for these compounds appear to be photolysis or photooxidation in the atmosphere and photooxidation and radical oxidation in water. Biodegradation may occur, but most studies show this to be a slow process. No information was available concerning the fate of the phenylenediamines in soil; however, leaching is expected to be important.

3. EXPOSURE

Limited information was found in the available literature as cited in Appendix A concerning exposure to the three phenylenediamines; no STORET information was found. Sutta et al. (1981) reported that ~15 million individuals are potentially exposed each year to a number of substituted phenylenediamines, including m- and p-phenylenediamine, which are used in the manufacture of hair dye either as a direct ingredient or as a synthetic intermediate. m-Phenylenediamine and p-phenylenediamine are also used as photographic developing agents, and potential human exposure also results from this use.

Environmental release and/or exposure information, however, is sparse. p-Phenylenediamine is used as a vulcanization accelerator and as an antioxidant in rubber compounds. No leaching of this compound from rubber gaskets used in sewer pipe joint mains after 8 years was reported by Cuirdell and Mulcock (1976). No information was available indicating whether or not the phenylenediamines are immobile when used as dyes or dye intermediates in plastics or antiozonates in rubber products (Federal Register, 1982), although it appears that these compounds do not leach readily from rubber (Hollifield and Lombardo, 1975).

4. PHARMACOKINETICS

4.1. ABSORPTION

Pertinent data regarding absorption following oral administration of the phenylenediamines were not located in the available literature as cited in Appendix A. The dermal absorption of p-phenylenediamine as a hair dye component has been investigated in monkeys and male volunteers (Maibach and Wolfram, 1981). Ring-labeled [^{14}C]-p-phenylenediamine-enriched "Blue Blacks Nice 'n Easy" (203 µCi/mg), containing 2.7% p-phenylenediamine, was applied at the rate of 42 µCi to the scalp hair of monkeys and at the rate of 564-786 µCi to the scalp of men. After dying, rinsing and thoroughly drying the hair, the hair was shaved from the scalp. Urine was collected from the experimental subjects for 7 days. Monkey hair retained radioactivity equivalent to 12.9-14.5% of the total dose of applied radioactivity. Somewhat higher percentages, 14.1-26.5%, were retained in human hair. Over the 7-day urine collection period, monkeys excreted radioactivity equivalent to 0.083-0.190% of the applied dose, and the volunteers excreted radioactivity equivalent to 0.072-0.207% of the applied dose. These data indicate that dermal absorption of p-phenylenediamine occurs during the use of hair dyes containing this chemical, although the rate or extent of absorption cannot be estimated from this study. Kiese et al. (1968) found that both m- and p-phenylenediamine were absorbed through the skin of dogs. Within hours, unchanged parent compound was found in the urine of dermally-treated dogs. Mixing the phenylenediamine with hydrogen peroxide before application reduced absorption.

4.2. DISTRIBUTION

Pertinent data regarding the distribution of the phenylenediamines were not located in the available literature as cited in Appendix A.

4.3. METABOLISM

Watanabe et al. (1976) injected o- or p-phenylenediamine at 100 µmol/kg bw intraperitoneally into male rats to study the production of diazo-positive metabolites as qualitative and quantitative indication of exposure to these phenylenediamine isomers. Urine was collected from treated rats for 5 hours following treatment, and was subjected to diazo-coupling by the Bratton-Marshall's reaction prior to colorimetric measurement. Treatment of rats with p-, but not o-phenylenediamine, resulted in the production of two diazo-positive metabolites in sufficient quantity to be colorimetrically measurable. These metabolites were not further quantitated or identified. Data regarding the metabolism of m-phenylenediamine were not located in the available literature as cited in Appendix A.

4.4. EXCRETION

The urinary excretion of radioactivity following dermal application of [^{14}C]-phenylenediamine has been discussed in Section 4.1. No other pertinent data regarding the excretion of the isomers of phenylenediamine were located in the available literature as cited in Appendix A.

5. EFFECTS

5.1. CARCINOGENICITY

Long-term oral carcinogenicity bioassays have been performed in rats and mice using each isomer of phenylenediamine. Also, several long-term dermal carcinogenicity bioassays have been performed with the m- and p-isomers, and with hair dye products containing the m- or p-isomers or mixtures of the two. In this section, the chronic oral studies will be discussed first, beginning with the o-, then the m- and the p-isomer. The dermal studies will follow the oral studies.

Weisburger et al. (1978) investigated the carcinogenicity of o-phenylenediamine, administered as o-phenylenediamine dihydrochloride, in rats and mice. Groups of 25 male and 25 female CD1-HaM/ICR mice and 25 male Sprague-Dawley rats were fed diets containing 0 (control), the MTD or half the MTD of this isomer based on short-term feeding studies previously performed. Treatment continued for 18 months. Following the feeding period, rats were held for an additional 6-month, and mice for an additional 3-month observation period, during which basal diets were fed. Male rats treated with o-phenylenediamine dihydrochloride received diets containing 2000 or 4000 ppm for the entire 18-month treatment period. Mice treated with the o-isomer received diets containing 4000 or 8000 ppm for the first 5 months and 8000 or 16,000 ppm for the last 13 months. The resultant TWA dietary concentrations for mice treated with o-phenylenediamine dihydrochloride were 6889 or 13,778 ppm. Animals that died during the first 6 months of the study were discarded without necropsy. Complete necropsy was performed on all other animals that died or were killed at termination. o-Phenylenediamine dihydrochloride appeared to be carcinogenic in both rats and mice, as evidenced by an increased incidence of hepatocellular carcinomas in both

species (Table 5-1). A significantly increased incidence of hepatocellular carcinomas was observed in high dose group male rats and mice, and female mice of both treated groups.

Using an identical protocol, Weisburger et al. (1978) studied the carcinogenicity of m-phenylenediamine in these strains of rats and mice. The male rats received diets containing 1000 or 2000 ppm m-phenylenediamine and mice received diets containing 2000 or 4000 ppm m-phenylenediamine throughout the entire 18-month feeding period. Extensive histopathological examination of a wide range of tissues revealed that m-phenylenediamine appeared to be without tumorigenic activity in the rats and mice in this study.

An NCI (1979) bioassay with p-phenylenediamine dihydrochloride was performed in rats and mice. Groups of 50 male and 50 female 6-week-old Fischer 344 rats and 50 male and 50 female 6-week-old B67C3F_1 mice were fed diets containing 625 or 1250 ppm p-phenylenediamine dihydrochloride. Control groups of 20 male and 20 female rats and 20 male and 20 female mice were maintained on basal diet without added p-phenylenediamine dihydrochloride. Treatment continued for 103 weeks, followed by an observation period of 2 weeks during which basal ration was fed.

High dose male and female rats of both treatment groups experienced a slight depression in mean body weights, compared with controls, indicating that the high dose may have been near the MTD (NCI, 1979). Surviving to termination were 13/20 (65%), 38/50 (76%) and 34/50 (68%) for male rats and 14/20 (70%), 39/50 (78%) and 39/50 (78%) for female rats in the control, low and high dose groups, respectively. Mortality of rats appeared not to be affected by treatment, and adequate numbers of rats survived sufficiently long to be at risk from late developing tumors. Extensive histopathological

TABLE 5-1

Incidences of Hepatocellular Carcinomas in Rats and Mice Fed Diets Containing o-Phenylenediamine Dihydrochloride for 18 Months[a]

	Matched Controls	Pooled Controls	Low Dose Group	High Dose Group
Male rats	0/16	2/111	0/14	5/16[b]
Male mice	0/14	7/99	5/17[b]	3/14
Female rats	1/15	1/102	6/18[c]	6/15[b]

[a]Source: Weisburger et al., 1978

[b]$p<0.025$ compared to incidence in matched and pooled controls

[c]$p<0.025$ compared to incidence in pooled controls

examination revealed that a variety of neoplasms occurred with similar frequency in both treated and control rats. These included leukemia or malignant lymphoma, pituitary tumors, testicular and lung tumors in males, and mammary and uterine tumors in females. Lung tumors in females (0/20, 1/49, 4/48 in control, low and high dose groups, respectively) and thyroid tumors in males (0/19, 2/48, 3/44 in control, low and high dose groups, respectively) occurred only in treated groups, but at low incidences that were not statistically significant.

Mean body weights of treated female but not male mice were slightly lower than those of controls (NCI, 1979). Surviving to termination were 16/20 (80%), 38/50 (76%) and 42/50 (84%) for male mice and 17/20 (85%), 44/50 (88%) and 41/49 (84%) for female mice in the control, low and high dose group, respectively. Mortality appeared not to be affected by treatment; adequate numbers of mice survived sufficiently long to be at risk from late developing tumors. Extensive histopathological examination showed that tumors of the lungs, hematopoietic system and liver occurred at a slightly higher, but not statistically significant, incidence in treated compared with control mice.

NCI (1979) concluded that under the conditions of this bioassay, p-phenylenediamine dihydrochloride was not carcinogenic to rats or mice.

The carcinogenicity of dietary p-phenylenediamine in rats was also studied by Imaida et al. (1983). Groups of 63-66 male and 63-66 female 6-week-old F344 rats were fed diets containing 500 or 1000 ppm p-phenylenediamine for 80 weeks. All survivors were killed at 80 weeks and subjected to histopathological examination. Controls consisted of 24-25 rats of each sex. Treatment appeared to have no effect on mortality. High dose females had body weights consistently below those of controls throughout the study.

Extensive histopathological examination of a wide range of tissues revealed that adrenal pheochromocytomas were the predominant tumor and occurred with similar frequency in control and treated rats. The incidence of the neoplastic lesions was not significantly greater in treated than control groups, and the authors concluded that p-phenylenediamine was not carcinogenic to rats in this study.

IARC (1978) mentioned an early Japanese study with p-phenylenediamine in which rats (age, sex and strain unspecified) were treated with 0, 0.06, 0.3, 10 or 30 mg/kg/day for up to 8 months (Saruta et al., 1958). Group size varied from four to five rats. Within 4 months, 3/4 of the high dose rats died. Mortality data were not reported for the other groups. Tumors were not observed in any treatment or control rats.

Holland et al. (1979) applied m-phenylenediamine to the shaved dorsal skin of groups of 40 male and 40 female C3Hf/Bd and 20 male and 20 female C57Bl/6Bd mice for 24 months. Dosages of 0.6 or 3 mg/mouse/week were divided and administered on Monday, Wednesday and Friday. Appropriate negative (acetone vehicle) and positive (benzo[a]pyrene) controls were maintained. Tumor incidence fluctuated widely, but was not correlated to dose or did not differ significantly from that of negative controls. Holland et al. (1979) concluded that m-phenylenediamine was not carcinogenic to either strain of mice in this study.

A dermal carcinogenicity study with p-phenylenediamine was performed in female Swiss mice and female rabbits (Stenback et al., 1977). Groups of 50 mice and 5 rabbits were treated twice weekly with 0.02 mℓ applications of 5 or 10% p-phenylenediamine for up to 85 weeks (rabbits) or until spontaneous death (mice). Appropriate vehicle (negative) and positive controls were maintained. Infectious diseases seemed to cause most of the deaths in both mice and rabbits. Survival appeared to be unaffected by treatment.

No increase in the incidence of dermal or internal tumors was observed in treated mice or rabbits compared with vehicle controls; positive controls responded appropriately. Stenback et al. (1977) concluded that p-phenylenediamine was not carcinogenic to mice or rabbits in this study.

Burnett et al. (1975) applied doses of hair dyes containing 0.17% m-phenylenediamine with or without 1.5% p-phenylenediamine to the shaved skin of Swiss Webster mice. Application was once weekly or every other week for 18 months. Negative controls (untreated and vehicle) and positive controls were maintained. There was no evidence that the hair dyes used in this study were carcinogenic to mice.

IARC (1978) mentioned several other skin painting studies in mice with hair dye formulations containing m- or p-phenylenediamine or both. The incidence of tumors in treated mice was no higher than that in control mice, which were treated with the dye base without phenylenediamines.

IARC (1978) also reported that subcutaneous injection of m-phenylenediamine (Saruta et al., 1962) or p-phenylenediamine (Saruta et al., 1958) resulted in the development of local injection site sarcomas in rats..

5.2. MUTAGENICITY

The mutagenicity and genotoxicity of the isomers of phenylenediamine have been studied extensively in several different assays and indicator systems. To clarify the large amount of data, the information is summarized in tables primarily by assay and secondarily by isomer. Tables 5-2 through 5-4 summarize data from reverse mutation studies in prokaryotes, a forward mutation study in Klebsiella and mouse lymphoma cells and a mitotic recombination study in Saccharomyces cerevisiae. Dominant lethal, sex-linked recessive lethal and mouse spot tests are summarized in Table 5-5. Chromosomal aberrations tests and other tests of genotoxicity are summarized in Table 5-6.

TABLE 5-2

Summary of Reverse Mutation and Forward Mutation Studies with the o-Isomer of Phenylenediamine

Assay	Indicator Organism	Application	Concentration or Dose	Activating System	Response	Comments	Reference
Reverse mutation	Salmonella typhimurium TA1538	plate incorporation	0-100 µg/plate	±S-9	±	NC	Ames et al., 1975
Reverse mutation	S. typhimurium TA1538, G46, TA1535 TA100, TA98, C3076 TA1537, D3052	plate incorporation	0-1000 µg/mL	±S-9	±	10-fold concentration gradient/ plate. Positive in TA1535, D3052, TA1538, TA98.	Thompson et al., 1983
Reverse mutation	S. typhimurium TA1538	plate incorporation	0.50 or 100 µg/plate	±S-9	±	NC	Garner and Nutman, 1977
Reverse mutation	S. typhimurium TA98, TA100, TA1537	plate incorporation	0.03-15 µg/plate	±S-9	±	Positive only in strain TA98.	Voogd et al., 1980
Reverse mutation	S. typhimurium TA98, TA100, TA1537	plate incorporation	NR	S-9		Positive only in strain TA98.	Ishidate et al., 1981; Ishidate and Yoshikawa, 1980
Reverse mutation	S. typhimurium TA1538	plate incorporation	0-100 µg/plate	S-9	+	Treatment with H_2O_2 before plating increases magnitude of positive response.	Venitt and Searle, 1976
Reverse mutation	S. typhimurium TA1538	plate incorporation	10 µg/plate	S-9	+	Treating rats and mice with beta-naphthoflavone enhances activity of S-9.	Dybing and Thorgeirsson, 1977
Reverse mutation	S. typhimurium TA98	plate incorporation	1 µmol/plate	liver components	+	Microsomal inhibitors inhibit activity of S-9.	Nohmi et al., 1982
Reverse mutation	S. typhimurium TA98	plate incorporation	5 µmol/plate	S-9	+	3-Methylcholanthrene enhances activity of S-9.	Yoshikawa et al., 1979
Reverse mutation	S. typhimurium TA1535	plate incorporation	1 µg/plate	none	±	Mutagenic activity ratio = 1:1.	Commoner, 1976
Reverse mutation	Escherichia coli WP2, WP2uvrA⁻	plate incorporation	0-1000 µg/mL	±S-9	±	Positive only in strain WP2.	Thompson et al., 1983
Forward mutation fluctuation test	Klebsiella pneumoniae	liquid incubation	1.5 µg/tube	none	-	NC	Voogd et al., 1980
Forward mutation	Mouse lymphoma L5178Y	cell culture	NR	S-9	+	NC	Thompson, 1981

NC = No comment; NR = Not reported

TABLE 5-3

Summary of Reverse Mutation and Forward Mutation and Mitotic Recombination Studies with the m-Isomer of Phenylenediamine

Assay	Indicator Organism	Application	Concentration or Dose	Activating System	Response	Comments	Reference
Reverse mutation	Salmonella typhimurium TA1538	plate incorporation	0-100 µg/plate	±S-9	±	NC	Ames et al., 1975
Reverse mutation	S. typhimurium TA1538, G46, TA1535, TA100, TA98, C3076 TA1537, D3052	plate incorporation	0-1000 µg/mL	±S-9	±	Ten-fold concentration gradient/plate. Positive in TA1535, D3052, TA1538, TA98.	Thompson et al., 1983
Reverse mutation	S. typhimurium TA1538	plate incorporation	0.50 or 100 mg/plate	±S-9	±	NC	Garner and Nutman, 1977
Reverse mutation	S. typhimurium TA98, TA100, TA1537	plate incorporation	NR	S-9		Positive only in strain TA98.	Ishidate et al., 1981; Ishidate and Yoshikawa, 1980
Reverse mutation	S. typhimurium TA1538	plate incorporation	0-100 µg/plate	S-9	+	Treatment with H_2O_2 before plating increases magnitude positive response.	Venitt and Searle, 1976
Reverse mutation	S. typhimurium TA1538	plate incorporation	10 µg/plate	S-9	+	Treating rats and mice with beta-naphthoflavone enhances activity of S-9.	Dybing and Thorgeirsson, 1977
Reverse mutation	S. typhimurium TA98	plate incorporation	1 µmol/plate	liver components	+	Microsomal inhibitors inhibit activity of S-9.	Nohmi et al., 1982
Reverse mutation	S. typhimurium TA98	plate incorporation	5 µmol/plate	S-9	+	3-Methylcholanthrene enhances activity of S-9.	Yoshikawa et al., 1979
Reverse mutation	S. typhimurium TA1538, TA98, TA100	plate incorporation	5-1000 µg/plate	±S-9	±	Positive in TA98, TA1538 with or without purification by purification by distillation.	Shahin et al., 1980
Reverse mutation	S. typhimurium TA1538, TA98, TA100	plate incorporation	0-100 µg/plate	±S-9	±	Weakly positive in TA1538, TA98; negative in TA100	de Giovanni-Donnelly, 1981
Reverse mutation	S. typhimurium TA1538, TA98	plate incorporation	20-1000 µg/plate	±S-9	‡	Response weakly positive without S-9; strongly positive with S-9.	Picciano et al., 1983

TABLE 5-3 (cont.)

Assay	Indicator Organism	Application	Concentration or Dose	Activating System	Response	Comments	Reference
Reverse mutation	Escherichia coli WP2, WP2uvrA⁻	plate incorporation	0-1000 µg/mL	±S-9	±	Positive only in strain WP2	Thompson et al., 1983
Mitotic recombination	Saccharomyces cerevisiae D3, D4	spot tests, plate incorporation, liquid incubation	≤500 µg/mL	±S-9	-	NC	Mayer and Goin, 1980

NC = Not reported; NR = Not reported

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TABLE 5-4

Summary of Reverse Mutation and Forward Mutation Studies with the p-Isomer of Phenylenediamine

Assay	Indicator Organism	Application	Concentration or Dose	Activating System	Response	Comments	Reference
Reverse mutation	Salmonella typhimurium TA1538	plate incorporation	0-100 µg/plate	±S-9	-	Component of hair dye; mutagenic only when mixed with H_2O_2.	Ames et al., 1975
Reverse mutation	S. typhimurium TA1538, G46, TA1535, TA100, TA98, C3076, TA1537, D3052	plate incorporation	0-1000 µg/ml	±S-9	±	Ten-fold concentration gradient/plate. Positive only in strain TA98.	Thompson et al., 1983
Reverse mutation	S. typhimurium TA1538	plate incorporation	0, 50 or 100 µg/plate	±S-9	±	NC	Garner and Nutman, 1977
Reverse mutation	S. typhimurium TA98	plate incorporation	20 µg/plate	+S-9	+	Compound exposed to light for 0-4 hours. Dose-related increase revertants/plate for 0-1 hours.	Nishi and Nishioka, 1982
Reverse mutation	S. typhimurium TA1538	plate incorporation	0-100 µg/plate	S-9	+	Treatment with H_2O_2 before plating increases magnitude of positive response.	Venitt and Searle, 1976
Reverse mutation	S. typhimurium TA1538	plate incorporation	10 µg/plate	S-9	+	Treating rats and mice with β-naphthoflavone enhances activity of S-9.	Dybing and Thorgeirsson, 1977
Reverse mutation	S. typhimurium TA98	plate incorporation	5 µmol/plate	liver components	+	Microsomal inhibitors inhibit activity of S-9.	Nohmi et al., 1982
Reverse mutation	S. typhimurium TA98	plate incorporation	5 µmol/plate	S-9	+	3-Methylcholanthrene enhances activity of S-9.	Yoshikawa et al., 1979
Reverse mutation	S. typhimurium TA98	plate incorporation	0.25-2.0 mg/plate 0.25-2.0 mg/plate	±S-9 ±S-9	- ±	Purified isomer. Commercially available from two sources.	Crebelli et al., 1981
Reverse mutation	S. typhimurium TA98, TA1538	plate incorporation	25-250 µg/plate	S-9	+	Positive results obtained with DMSO, not water solutions. Number of revertants/plate proportional to time solution stands before plating.	Burnett et al., 1982

TABLE 5-4 (cont.)

Assay	Indicator Organism	Application	Concentration or Dose	Activating System	Response	Comments	Reference
Reverse mutation	S. typhimurium TA98, TA1538, TA1535, TA100, TA1537	plate incorporation	5-1000 µg/plate	S-9	+	Slightly increased activity with Aroclor-induced compared with uninduced rat S-9 TA98, TA1538. Oxidation with H_2O_2 did not enhance mutagenicity.	Shahin et al., 1979
Reverse mutation	S. typhimurium TA98, TA100	plate incorporation	0.5-2.0 µmol/plate	±S-9	±	Positive in TA98 only.	Degawa et al., 1979
Reverse mutation	S. typhimurium TA98	plate incorporation	≤25 mg dye/plate	±S-9	±	Various hair dyes, some containing up to 3% p-phenylenediamines. 12 of 25 dyes gave positive response.	Albano et al., 1982
Reverse mutation	Escherichia coli WP2, WP2uvrA⁻	plate incorporation	0-1000 µg/mL	±S-9	±	Positive only in strain WP2.	Thompson et al., 1983

NC = No comment

TABLE 5-5

Summaries of Dominant Lethal, Sex-Linked Recessive Lethal and Mouse Spot Test with o-, m- and p-Phenylenediamine

Assay	Indicator Organism	Isomer	Application	Concentration or Dose	Activating System	Response	Comments	Reference
Mouse spot test	mouse	o-phenylene-diamine	i.p.	176 mg/kg bw	NA	-	Single dose on day 10 of gestation; day of vaginal plug = day 1.	Gocke et al., 1983
Dominant-lethal test	rat	o-phenylene-diamine	i.p.	20 mg/kg bw	NA	-	Dose administered 3x weekly for 8 weeks, then males were mated to females.	Burnett et al., 1977
		m-phenylene-diamine	i.p.	20 mg/kg bw	NA			
		p-phenylene-diamine	i.p.	20 mg/kg bw	NA	-		
Dominant-lethal	rat	m-phenylene-diamine	i.p. 3x weekly for 10 weeks	12.5 mg/kg/dose	NA	±	Weakly positive; negative on retest.	Sheu and Green, 1979
Sex-linked recessive-lethal	Drosophila melanogaster	p-phenylene-diamine	adult feeding	5.1-15.5 mmol/ml	NA	+	Weakly positive.	Blijleven, 1977
Sex-linked recessive-lethal	D. melanogaster	p-phenylene-diamine	injection or adult feeding	2.5-15.5 mmol/ml	NA	-	Injection lethal to many flies. Negative results with more purified compound than previous experiment.	Blijleven, 1977, 1981

i.p. = intraperitoneal

NA = Not applicable

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Summaries of Chromosomal Aberration Tests and Other Tests of Genotoxicity with the o-, m- and p-Isomers of Phenylenediamine

Assay	Indicator Organism	Isomer	Application	Concentration or Dose	Activating System	Response	Comments	Reference
Chromosomal aberration	Chinese hamster lung fibroblasts	o-phenylenediamine	cell culture	≤50% growth inhibition dose	±S-9	‡	o-Phenylenediamine ~10-fold as potent as m-phenylenediamine; chromatid breaks, exchanges and ring formations most commonly observed.	Ishidate, et al., 1981; Ishidate and Yoshikawa, 1980
		m-phenylenediamine	cell culture	≤50% growth inhibition dose	±S-9	‡		
Chromosomal aberration	Syrian golden hamster embryo fibroblasts	m-phenylenediamine	cell culture	0.05-50 µg/mℓ	±S-9	‡	Lowest transforming dose = 5.0 µg/mℓ. Purified sample gave negative results.	Pienta, 1980a,b; Pienta and Kawalek, 1981
Chromosomal aberration Micronucleus test	mouse	o-phenylenediamine	i.p.	2 doses, 27-324 mg/kg bw	NA	+	Positive at 108-324 mg/kg, bone marrow cells.	Wild et al., 1980
	mouse	o-phenylenediamine	oral	2 doses, 108-324 mg/kg bw	NA	+	Positive at 108-324 mg/kg, bone marrow cells.	
	Chinese hamster	o-phenylenediamine	i.p.	2 doses, 54-324 mg/kg bw	NA	+	Positive at 216-324 mg/kg, bone marrow cells.	
	guinea pig	o-phenylenediamine	i.p.	2 doses, 108-324 mg/kg bw	NA	+	Positive at 108-324 mg/kg, bone marrow cells.	
Chromosomal aberration Micronucleus test	CFY rats	p-phenylenediamine	gavage	300 mg/kg bw	NA	-	Erythrocytes examined for micronuclei	Hossack and Richardson, 1977
Unscheduled DNA synthesis	rat hepatocyte	o-phenylenediamine	cell culture	0.5-1000 nmol/mℓ	NA	+	NC	Thompson et al., 1983
		m-phenylenediamine	cell culture	0.5-1000 nmol/mℓ	NA	-	NC	
		p-phenylene-	cell culture	0.5-1000 nmol/mℓ	NA	-	NC	
Unscheduled DNA synthesis	rat hepatocyte	p-phenylenediamine 2 HCl	cell culture	0.005-0.1 mg/mℓ	NA	-	NC	Williams et al., 1982
Cell survival assay	rat embryo 2FR$_4$50 cells	p-phenylenediamine	liquid incubation	1.85-3.2 µg/ 5.2 x 10^4 cells	none	+	Loss of anchorage dependence, manifested by increased survival considered a characteristic of neoplastic transformation.	Traul et al., 1981

TABLE 5-6 (cont.)

Assay	Indicator Organism	Isomer	Application	Concentration or Dose	Activating System	Response	Comments	Reference
Inhibition of testicular DNA synthesis	male mouse	o-phenylene-diamine	oral	200 mg/kg bw	NA	+	p-isomer inhibits testicular DNA synthesis > m-isomer > o-isomer.	Seiler, 1977
		m-phenylene-diamine	oral	200 mg/kg bw	NA	+		
		p-phenylene-diamine	oral	200 mg/kg bw	NA	+		
Sperm head abnormality	mouse spermatozoa	p-phenylene-diamine	5 daily i.p. injections	5-100 mg/kg bw/day	NA	-	CBAXBALB/CF1 male mouse spermatogenesis is particularly sensitive to mutagens.	Topham, 1980a,b

i.p. = intraperitoneal

NA = Not applicable; NA = No comment

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Ames et al. (1975) applied their reverse mutation assay in Salmonella typhimurium to the chemicals found in commercially available hair dyes, and observed that o- and m-phenylenediamine chemicals in this group yielded a positive response in S. typhimurium only after metabolic activation. p-Phenylenediamine gave a positive response only after oxidation with hydrogen peroxide, the normal procedure employed when using this chemical as a "permanent" hair dye. Garner and Nutman (1977), however, observed positive results with o-, m- and p-phenylenediamine in S. typhimurium with metabolic activation without exposure of the p-phenylenediamine to an external oxidizing system. Shahin et al. (1979) found that oxidation of p-phenylenediamine with H_2O_2 before plating did not increase the magnitude of the positive response in S. typhimurium, but Venitt and Searle (1976) observed an increased magnitude of the positive response with o-, m- and p-phenylenediamine in S. typhimurium when mixed with H_2O_2 prior to plating. By itself, H_2O_2 did not induce mutations in S. typhimurium (Ames et al., 1975; Venitt and Searle, 1976). Using S. typhimurium, Nishi and Nishioka (1982) observed an increase in the mutagenicity of p-phenylenediamine that was roughly proportional to duration of exposure to light. The system was metabolically activated.

Several other investigators (Thompson et al., 1983; Voogd et al., 1980; Venitt and Searle, 1976; Dybing and Thorgeirsson, 1977; Nohmi et al., 1982, Yoshikawa et al., 1979; Ishidate et al., 1981; Commoner, 1976; Burnett et al., 1977; DeGiovanni-Donnelly, 1981; Picciano et al., 1983) obtained positive results with the phenylenediamines in S. typhimurium. Generally, metabolic activation was necessary for a positive response. In most cases, it appeared that the m-isomer was considerably more mutagenic than the o- or p-isomers, which exhibited similar degrees of mutagenicity. An enhanced

mutagenicity with all three isomers was observed when S-9 that was harvested from animals pretreated with known enzyme-inducing agents was compared to S-9 from nonpretreated animals (Dybing and Thorgeirsson, 1977; Shahin et al., 1979; Yoshikawa et al., 1979). Adding microsomal inhibitors, 7,8-benzoflavone or SKF525-A to the S-9 before plate incorporation resulted in reduced mutagenic response of S. typhimurium to all three isomers (Nohmi et al., 1982).

In a reverse mutation test with Escherichia coli, o-, m- and p-phenylenediamine gave a positive response, but only with metabolic activation (Thompson et al., 1983). Negative results in a forward mutation fluctuation test in Klebsiella pneumoniae were observed by Voogd et al. (1980). Metabolic activation was not used in this test. In a forward mutation test, o-phenylenediamine was found to induce mutation of mouse L5178Y lymphoma cells to thymidine dependency (Thompson et al., 1981).

o-Phenylenediamine was negative in a mouse spot test (Gocke et al., 1983), and the three isomers were negative (Burnett et al., 1977; Sheu and Green, 1979) in dominant lethal tests in rats. Conflicting results were obtained with p-phenylenediamine in sex-linked recessive lethal tests in Drosophila melanogaster (Blijleven, 1977, 1981).

The o- and m-isomers of phenylenediamine have been strongly positive in chromosomal aberration tests (Ishidate et al, 1981; Ishidate and Yoshikawa, 1980; Pienta, 1980a,b; Pienta and Kawalek, 1981), with or without metabolic activation. The p-isomer was not tested in these experiments. Positive results in the micronucleus test were observed in several laboratory animals with o-phenylenediamine (Wild et al., 1980), but not with p-phenylenediamine (Hossack and Richardson, 1977). In the unscheduled DNA synthesis test in

rat hepatocytes, only the o-isomer yielded positive results (Thompson et al., 1983; Williams et al., 1982). All three isomers were positive in an inhibition of testicular DNA synthesis test in male mice (Seiler, 1977), and the p-isomer was positive in a cell-survival assay in rat embryo cells (Traul et al., 1981). p-Phenylenediamine was negative in a mouse sperm head abnormality test (Topham, 1980a,b).

5.3. TERATOGENICITY

Two studies of the teratogenicity of m-phenylenediamine were located, as well as one study with hair dyes containing small amounts (≤3%) of various combinations of the three isomers. No studies of the teratogenicity of "pure" o- or p-phenylenediamine were located in the available literature.

Picciano et al. (1983) treated mature female Sprague-Dawley rats by gavage with m-phenylenediamine at 0 (propylene glycol vehicle control), 45, 90 or 180 mg/kg bw/day on days 6-15 of gestation. The day that a vaginal plug or spermatozoa in the vaginal smear was found was designated day 0 of gestation. Positive controls (vitamin A and aspirin) were maintained. Pregnant dams (22, 9, 8 and 7 in vehicle control, 45, 90 and 180 mg/kg bw/day groups, respectively) were killed and reproductive tracts and contents were examined on gestation day 20. No deaths occurred, and no signs of maternal toxicity were observed, except that high dose rats exhibited a significantly ($p<0.05$) reduced rate of body weight gain. Parameters of fertility and fetal body weights were unaffected by treatment. The incidences of gross external skeletal and visceral anomalies were not increased by exposure to m-phenylenediamine. Positive controls responded appropriately.

A German paper reported the results of a teratogenicity study of m-phenylenediamine in rats (Hruby et al., 1981). Groups of 25 mated female rats were treated by gavage with m-phenylenediamine at 0 (water control), 10, 30 or 90 mg/kg bw/day on days 6-15 of gestation. In addition, a positive control group was treated with acetylsalicylic acid. Six high dose rats died; no rats in the other groups died. Evidence of fetotoxicity was noted in high dose rats; a smaller number of litters with live fetuses, reduced placental weight, fewer living fetuses/litter, reduced fetal body weights, increased incidence of fetal resportion and a greater number of dead fetuses were observed. Major fetal malformations were not observed in treated rats, but an increase in the percentage of litters with minor fetal abnormalities and a greater percentage of fetuses with minor abnormalities were noted in high dose rats, compared with negative controls. These signs of fetotoxicity and fetal anomalies were not statistically significant in the 10 or 30 mg/kg bw/day dosed groups.

Burnett et al. (1976) studied the teratogenicity of 12 hair dye formulations in rats. Of these formulations, one contained 3.0 and another contained 2.0% p-phenylenediamine. A third formulation contained 1.0% o-phenylenediamine and 1.5% m-phenylenediamine. All formulations contained a potpourri of other chemicals and were made up to 100% with water. A dose of 2 mL/kg of dye formulation was applied to the shaved backs of groups of 20 mated female Charles River CD rats on gestation days 1, 4, 7, 10, 13, 16 and 19 (vaginal smear positive for spermatozoa = gestation day 1). In addition, three groups of 20 negative controls were maintained. They were shaved but not treated. A positive control group was treated with acetylsalicylic acid by gavage at 250 mg/kg bw/day on days 6-16 of gestation. The rats were sacrificed and reproductive tracts and contents were examined on

gestation day 20. About one-third of the fetuses from each litter were examined for visceral anomalies, and two-thirds were examined for skeletal anomalies. Positive control rats responded appropriately. No signs of maternal toxicity changes in food consumption or body weights, or effects on reproductive parameters were observed in dye-treated rats compared with controls. No treatment-related effects on the incidence of soft tissue or skeletal abnormalities were noted.

5.4. OTHER REPRODUCTIVE EFFECTS

In a mutagenicity study in mice, Gocke et al. (1983) administered o-phenylenediamine at 176 mg/kg bw to pregnant mice on day 10 of gestation (Section 5.2.). Reduced litter size at birth and at weaning were noted. No other studies of reproductive effects associated with the phenylenediamines were located.

5.5. CHRONIC AND SUBCHRONIC TOXICITY

Chronic and subchronic oral toxicity studies have been performed with each of the three isomers of phenylenediamine. In this section, the tests with the o- and m-isomers are discussed first, followed by tests with the p-isomer. Chronic toxicity studies are discussed before shorter-term studies.

Only one chronic oral study with the o- and m-phenylenediamines, the Weisburger et al. (1978) study in mice and rats (discussed in Section 5.1.), was located in the available literature as cited in Appendix A. Since this experiment was designed solely as a carcinogenicity bioassay, parameters of chronic toxiciy of o- and m-phenylenediamine were not reported.

Weisburger et al. (1978) also mentioned a 30-day dose range-finding study using o- and m-phenylenediamine dihydrochloride in mice and rats, but the protocol and results of these studies were not available.

Chronic oral studies with p-phenylenediamine have been conducted by NCI (1979) and Imaida et al. (1983). In the NCI (1979) bioassay, groups of 50 male and 50 female 6-week-old Fischer 344 rats and B6C3F1 mice were fed diets containing 625 or 1250 ppm p-phenylenediamine dihydrochloride for 103 weeks, followed by a 2-week observation period during which basal diet was fed. Control groups consisted of 20 male and 20 female rats and mice maintained on basal diet for 105 weeks. High dose male rats and female rats in both treated groups experienced a slight depression in mean body weights, compared with controls. Among mice, mean body weights of females in both treatment groups were slightly reduced, compared with controls. Body weights of male mice were unaffected by treatment. No other clinical signs of toxicity were observed in either rats or mice, and mortality was unaffected by treatment. Upon extensive histopathological examination of a wide range of tissues, degenerative, inflammatory and proliferative lesions occurred at similar frequency in treated and control rats and mice; which were not considered to be related to treatment.

Imaida et al. (1983) fed diets containing 0 (control), 500 or 1000 ppm p-phenylenediamine to 6-week-old F344 rats for 80 weeks. The treated groups consisted of 63-66 males and 63-66 females/group. The controls consisted of 24 males and 25 females. Mortality rate and food consumption of treated rats of both sexes and body weights of males were unaffected by treatment. High dose females exhibited reduced body weights throughout the study compared with controls. No treatment-related effects on hematology, evaluated only at termination, on relative organ weights or on the histopathological appearance of a wide range of tissues were observed.

A subchronic dose range-finding study with p-phenylenediamine dihydrochloride was conducted in both rats and mice (NCI, 1979) carcinogenicity

bioassay. Groups of five male and five female Fischer 344 rats were fed diets containing 0, 68, 100, 147, 215, 316, 464, 681, 1000, 1470, 2150 or 3160 ppm of p-phenylenediamine dihydrochloride for 7 weeks, followed by a 1-week observation period during which basal diet was fed. All animals survived to termination. Arched backs and rough hair coats, clinical signs of toxicity, were observed only in the 3160 ppm dose rats of both sexes. Body weight gains of rats of both sexes at $\leq$681 ppm appeared to be unaffected by treatment. No other parameters of toxicity were studied.

Using the identical protocol, another subchronic feeding study was conducted in mice using dietary levels of 0, 100, 147, 215, 316, 464, 681, 1000, 1470, 2150, 3160 or 4640 ppm p-phenylenediamine dihydrochloride (NCI, 1979). No mortality occurred, and no clinical signs of toxicity were observed in any groups. Mean body weight gains appeared to be unaffected in mice receiving diets containing $\leq$1000 ppm p-phenylenediamine dihydrochloride.

Imaida et al. (1983) performed a subchronic oral toxicity test with p-phenylenediamine in groups of 10-11 male and 10-11 female 6-week-old F344 rats. Groups were fed diets containing 0, 500, 1000, 2000 or 4000 ppm p-phenylenediamine for 12 weeks, at which time survivors were killed and histopathologically examined. One high dose female and nine high dose male rats died before the completion of the experiment. No other deaths were reported. From graphs of body weights plotted throughout the experiment, it appeared that there was a dose-dependent reduction in the rate of body weight gain in rats of both sexes. Although statistical analysis was not performed, body weights of males and females in the 2000 and 4000 ppm groups were substantially below those of controls throughout the study. High dose rats of both sexes had substantially elevated relative liver and kidney weights, compared with controls.

An abstract of a Japanese study (Matsuoka, 1962) reported that rats subcutaneously injected with 0.1 times the subcutaneous LD_{50} in mice of o-phenylenediamine (45 mg/kg), m-phenylenediamine (9 mg/kg) or m-phenylenediamine dihydrochloride (12 mg/kg) every other day manifested decreased growth rate, a marked decrease in erythrocyte and leukocyte counts and decreased blood hemoglobin concentration. Apparently, histopathological lesions were observed in several organs.

5.6. OTHER RELEVANT INFORMATION

Acute LD_{50} values for the phenylenediamines are summarized in Table 5-7. Generally, it appears that the o-isomer is least toxic and the p-isomer is most toxic to rats. The acute oral toxicity syndrome in humans involves acute renal failure, according to Chugh et al. (1982) and Suliman et al. (1983), who reported cases of acute (suicidal attempts) ingestion.

Burnett et al. (1976) studied the dermal toxicity of 12 hair dye formulations in rabbits. Of the formulations tested, one contained 3.0% and another contained 2.0% p-phenylenediamine, and a third contained 1.5% m- and 1.0% o-phenylenediamine. Groups of six New Zealand white rabbits of each sex were treated with 1 mℓ of a 1:1 mixture of the dye and 6% hydrogen peroxide. Application was twice weekly for 13 weeks; half of the application sites were abraded. Treatment appeared to have no effect on rate of body weight gain, hematologic or clinical chemistry parameters, or gross or microscopic pathology.

In a dermal irritation test in rabbits, p-phenylenediamine was determined to be a mild irritant (Lloyd et al., 1977). In humans, the phenylenediamine isomers have not been implicated as skin irritants, but p-phenylenediamine has been associated with many cases of contact sensitization (Malten et al., 1973; Epstein and Taylor, 1979; Camarasa, 1979; Lachapelle and

TABLE 5-7

Acute LD_{50} Values for the Phenylenediamines (mg/kg)

Species	Route	o-Phenylene-diamine	o-Phenylene-diamine·2 HCl	m-Phenylene-diamine	m-Phenylene-diamine·2 HCl	p-Phenylene-diamine	p-Phenylene-diamine·2 HCl	Reference
Mouse	s.c.	450	NR	90	120	NR	NR	Matsuoka, 1962
Rat	oral	NR	NR	NR	NR	98	NR	Lloyd et al., 1977
Rat	oral	1070	NR	650	NR	80	NR	Burnett et al., 1977
Rat	oral	NR	NR	NR	NR	100	NR	Watanabe et al., 1976
Rat	i.p.	516	NR	283	NR	37	NR	Burnett et al., 1977
Rat	i.p.	NR	290	NR	325	NR	NR	Weisburger et al., 1978
Rat	sc	600	NR	NR	NR	NR	NR	Watanabe et al., 1976

s.c. = subcutaneous

i.p. = intraperitoneal

NR = Not reported

Tennstedt, 1979; Warshawshki et al., 1981; Kilpaikari, 1982). In guinea pigs, p-phenylenediamine induced a strong sensitization reaction in 90-100% of those tested by the SIAT, maximization or modified Draize methods (Goodwin et al., 1981). Using volunteers, Marzulli and Maibach (1974) demonstrated a dose-dependency to the magnitude of the allergic response to p-phenylenediamine. In an abstract of a Russian study, m-phenylenediamine was found to cause a dermal allergic response in rats, rabbits and guinea pigs (Lopatneva et al., 1978). The response was especially strong in guinea pigs. In an abstract, Gaul (1960) reported that human subjects sensitized to p-phenylenediamine did not cross-react to patch tests with the o- or m-isomers.

In other studies on the effects of the phenylenediamines, Watanabe et al. (1976) demonstrated that both o- and p-phenylenediamine caused conversion of hemoglobin to methemoglobin in vivo and in vitro in rats. The in vivo effect of the two isomers was nearly equivalent, but the p-isomer was about twice as potent as the o-isomer in vitro. Messner et al. (1979), studying the in vitro effects of aromatic amines on fatty acid and cholesterol synthesis in rat liver, observed that o-phenylenediamine moderately accelerated the synthesis of fatty acids, but not cholesterol, from [^{14}C]-acetate.

Jain et al. (1979) observed cataractous lenticular changes in the eyes of 89% of 200 individuals who had worked with hair dyes for <1 year. Only 23% of a "control" group exhibited similar lenticular changes. Similar changes were produced in the eyes of rats and rabbits treated with eye drops or subconjunctival injections of p-phenylenediamine-containing hair dyes.

Riley (1960) showed that o-, m- and p-phenylenediamine inhibited mouse melanoma tumor growth in vitro and in vivo, resulting in longer survival times.

6. AQUATIC TOXICITY

6.1. ACUTE EFFECTS

It was reported in an abstract of a Russian study that solutions of 1, 5 and 10 mg/ℓ p-phenylenediamine were toxic to 100% of Daphnia in 24 hours (Zhakov, 1960). The 1 and 5 mg/ℓ solutions were not toxic after they were allowed to stand for 5-6 days, but the 10 mg/ℓ solution was toxic to 50% of the Daphnia after 5-6 days.

6.2. CHRONIC EFFECTS

Pertinent data regarding the chronic effects of the phenylenediamines were not located in the available literature as cited in Appendix A.

6.3. PLANT EFFECTS

Pertinent data regarding the plant effects of the phenylenediamines were not located in the available literature as cited in Appendix A.

6.4. RESIDUES

Pertinent data regarding the effects of residues of the phenylenediamines were not located in the available literature as cited in Appendix A.

7. EXISTING GUIDELINES AND STANDARDS

A TWA-TLV for p-phenylenediamine of 0.1 mg/m³ is currently recommended by the ACGIH (1980, 1983) for workroom air. A STEL has not been recommended. The Occupational Safety and Health Administration has promulgated a TWA standard for of 0.1 mg/m³ p-phenylenediamine (CFR, 1981). It was noted by both of these agencies that absorption via the cutaneous route (skin) can significantly contribute to total exposure. No guidelines or standards for o- or m-phenylenediamine were located in the available literature.

8. RISK ASSESSMENT

Long-term oral carcinogenicity bioassays were conducted with o- and m-phenylenediamine dihydrochloride in rats and mice (Weisburger et al., 1978), p-phenylenediamine dihydrochloride in rats and mice (NCI, 1979), and p-phenylenediamine in rats (Imaida et al., 1983). These investigators concluded that m- and p-phenylenediamine dihydrochloride and p-phenylenediamine were not carcinogenic to these species in these studies. Weisburger et al. (1978) found, however, that a statistically significant increase in hepatocellular carcinomas occurred in both rats and mice fed diets containing o-phenylenediamine dihydrochloride for 18 months (see Table 5-1).

The mutagenicity of the phenylenediamines has been studied extensively in microorganisms. Generally, the o-, m- and p-isomers yielded positive responses in reverse mutation assays in prokaryotes with but not without metabolic activation (Ames et al., 1975; Thompson et al., 1983; Garner and Nutman, 1977; Nishi and Nishioka, 1982; Voogd et al., 1980; Ishidate et al., 1981; Venitt and Searle, 1976; Dybing and Thorgeirsson, 1977; Yoshikawa et al., 1979). o-Phenylenediamine was negative in the mouse spot test (Gocke et al., 1983), and all three isomers were negative in a dominant-lethal assay in rats (Burnett et al., 1977). The o-isomer, but not the p-isomer, was associated with DNA damage as evaluated by the unscheduled DNA synthesis test (Thompson et al., 1983).

No studies specifically designed to test the chronic toxicity of the phenylenediamine isomers were located in the available literature as cited in Appendix A. Limited chronic toxicity data were available, however, from the NCI (1979) bioassay in rats and mice, and the Imaida et al. (1983) study in rats. In the NCI (1979) study, high dose (1250 ppm) male rats and female

rats and mice in both treated groups experienced a slight depression in mean body weights compared with controls. Food intake data were not reported. No other clinical signs of toxicity were observed. Histopathological examination revealed no lesions associated with treatment with p-phenylenediamine dihydrochloride. In the Imaida et al. (1983) study with p-phenylenediamine in rats, the only adverse effect noted was depressed body weights in high dose (1000 ppm) females; food consumption was unaffected.

Weisburger et al. (1978) fed male rats and male and female mice diets containing o- or m-phenylenediamine dihydrochloride for 18 months. Chronic toxicity data were not reported for this study.

Carcinogenic potency (q_1^*) values for humans can be derived from the 18-month feeding studies in rats and mice by Weisburger et al. (1978). In these studies male rats were fed diets containing 2000 or 4000 ppm o-phenylenediamine dihydrochloride and male and female mice received TWA dietary concentrations of 6889 or 13,778 ppm o-phenylenediamine dihydrochloride. A significantly increased incidence of heptocellular carcinomas was observed in the high-dose group male rats and mice and female mice of both groups (see Table 5-1). The most appropriate data for the determination of a carcinogenic potency are those for the development of hepatocellular carcinomas in male rats. These data indicate a higher sensitivity to o-phenylenediamine in male rats as evidenced by the much lower doses required to induce hepatocellular carcinomas. Also, the data resulted in the derivation of the highest q_1^* value for humans. The data used for the calculation of the human q_1^* values from the studies in male rats, male mice and female mice are presented in Appendices B, C and D, respectively. The transformed doses calculated for male rats are based on the assumption that rats consume food at the rate of 5% of their body weight/day, and by expanding the length

of exposure (548 days) to the length of the experiment (730 days). The transformed doses calculated for male and female mice are based on the assumption that mice consume food equivalent to 13% of their body weight/day, and by expanding the length of exposure (548 days) to the length of the experiment (639 days). An adjustment has also been made to convert the transformed doses of o-phenylenediamine dihydrochloride to equivalent amounts of o-phenylenediamine. From these data, and using the linearized multistage model developed by Kenneth Crump and adopted by the U.S. EPA (Federal Register, 1980), human q_1^* value from the study on male rats was 4.68×10^{-2} $(mg/kg/day)^{-1}$. The conversion factor used in calculating q_1^* values for humans from animal q_1^* values was the cube root of the body weight ratio (Federal Register, 1980), which has been demonstrated empirically to adjust for species-to-species extrapolation. It should be noted that all three human q_1^* values are similar in magnitude.

There is sufficient evidence from the available carcinogenicity bioassay of o-phenylenediamine to regard the compound as a positive animal carcinogen following oral exposure. The complete absence of epidemiological studies on o-phenylenediamine, however, place this chemical in Class 2B in the IARC classification and B2 in the newly proposed EPA scheme.

m-Phenylenediamine dihydrochloride was not carcinogenic in the Weisburger et al. (1978) study in rats and mice. Furthermore, no toxicity data were reported for the rats or mice in this study. Since no other chronic or subchronic data were found regarding m-phenylenediamine, no data base exists from which to derive an ADI for this isomer.

p-Phenylenediamine and p-phenylenediamine dihydrochloride were not carcinogenic in rats and mice in two long-term studies (NCI, 1979; Imaida et al., 1983). No other chronic studies with p-phenylenediamine were located

in the available literature. Reduced rate of body weight gain was observed in all groups of treated rats and mice in the NCI (1979) study. Food consumption data were not reported. No other signs of toxicity were observed. In the Imaida et al. (1983) study, the only adverse sign reported was weight loss in high dose (1000 ppm) female rats. Food consumption was not affected by treatment. Assuming that rats consume food equivalent to 5% of their body weight/day, the low dietary concentration of 625 ppm p-phenylenediamine dihydrochloride in the NCI (1979) study corresponds to an intake of p-phenylenediamine dihydrochloride of 31.25 mg/kg/day, equivalent to p-phenylenediamine at 18.7 mg/kg/day. Since the slight reduction in body weight in treated female rats was observed without accompanying signs of toxicity or histopathological lesions, the dose of 18.7 mg/kg/day equivalent of p-phenylenediamine can be regarded as a NOAEL. Application of an uncertainty factor of 100, 10 for interspecies extrapolation and 10 to provide additional protection for unusually sensitive members of the population, results in a human ADI of 0.19 mg/kg/day or 13 mg/day for a 70 kg man.

9. REPORTABLE QUANTITY (RQ) RANKING BASED ON CHRONIC TOXICITY

No studies designed to investigate the chronic toxicity of the phenylenediamines were located in the available literature as cited in Appendix A. An 18-month feeding study with o- and m-phenylenediamine dihydrochloride was performed in both rats and mice (Weisburger et al., 1978) to investigate carcinogenicity, but chronic toxicity data were not presented. An NCI (1979) bioassay of p-phenylenediamine dihydrochloride in rats and mice provides a minimum of chronic toxicity data. Female rats and mice exhibited depressed mean body weights at the 625 ppm dietary concentration, the lowest dose tested, when compared with controls, but food consumption data were not reported. No other signs of toxicity were observed, and histopathological examination revealed no evidence of treatment-related lesions. In an 80-week study in rats, weight loss was observed in females fed diets containing 1000 ppm p-phenylenediamine (Imaida et al., 1983). Food consumption was equivalent to that of controls. No other signs of toxicity or treatment-related histopathological lesions were observed.

An RQ can be calculated only for p-phenylenediamine dihydrochloride by oral exposure based on the decreased mean body weight gain, compared with controls, observed in both rats (NCI, 1979; Imaida et al., 1983) and mice (NCI, 1979) fed diets containing $\geq$625 (rats) or 1250 ppm (mice) p-phenylenediamine dihydrochloride. Assuming rats eat food equivalent to 5% of their body weight/day, the lower dietary level of 625 ppm p-phenylenediamine dihydrochloride from the NCI (1979) study, corresponds to an animal MED of 18.7 mg/kg/day equivalent of p-phenylenediamine. Multiplying the animal MED by the cube root of the ratio of the body weight of rats (0.35 kg) to man (70 kg), yields an equivalent human MED of 3.20 mg/kg/day or 224.0 mg/day

for a 70 kg man. The RV_d associated with this human MED is 2.0. The decreased rate of body weight gain observed in female rats at this level, in the absence of histopathological alteration, is appropriately assigned an RV_e of 2. A CS of 4.0, the product of RV_d and RV_e, corresponds to an RQ of 5000. Similarly, an RQ can be calculated for rats in the Imaida et al. (1983) study, which exhibited weight loss at the 1000 ppm dietary level of p-phenylenediamine. Applying the same assumption as before, the 1000 ppm dietary level in rats is equivalent to a human MED of 8.5 mg/kg/day or 595 mg/day for a 70 kg man, which corresponds to an RV_d of 1.3. An RV_e of 2, associated with slight loss of body weight, when multiplied by 1.3, results in a CS of 2.6 and an RQ of 5000.

An RQ can also be calculated for the mice in the NCI (1979) study fed diets containing 625 or 1250 ppm p-phenylenediamine dihydrochloride, both groups of which exhibited slightly reduced mean body weights. Assuming mice consume food equivalent to 13% of their body weight/day, an animal MED of 48.5 mg/kg/day (equivalent p-phenylenediamine) is calculated from the lower dietary level, 625 ppm. Multiplying this animal MED by the cube root of the ratio of the body weight of mice (0.03 kg) to man (70 kg) results in an equivalent human MED of 3.7 mg/kg/day or 259.0 mg/day for a 70 kg man. A human MED of 259 mg/day is associated with an RV_d of 1.9; the RV_e, associated with slightly reduced rate of body weight gain in the absence of histopathological lesions, is 2, appropriately selected to be the same as for the rats in this study that experienced the same effects. A CS of 3.8, associated with an RQ of 5000 (the same as for the rats in this study) is calculated from the RV_d and RV_e. These CSs are summarized in Table 9-1. It should be noted that the reduced rate of body weight gain and, subsequently, reduced final body weights observed in both rats and mice in the

TABLE 9-1

Composite Scores for the Oral Toxicity of Phenylenediamine

Species	Dose/Exposure	Human MED (mg/day)	RV_d	Effect	RV_e	CS	RQ	Reference
Rat	625 ppm p-phenylenediamine in diet for 18 months: 18.7 mg p-phenylenediamine/kg/day	224.0	2.0	Decreased rate of body weight gain	2	4.0	5000	NCI, 1979
Rat/ female	1000 ppm p-phenylenediamine in diet for 80 weeks: 50 mg/kg/day	595	1.3	Slight loss of body weight	2	2.6	5000	Imaida et al., 1983
Mice	625 ppm p-phenylenediamine dihydrochloride in diet for 18 months: 48.5 mg p-phenyl-enediamine/kg/day	259	1.9	decreased rate of body weight gain	2	3.8	5000	NCI, 1979

NCI (1979) study occurred at 625 ppm dietary p-phenylenediamine dihydrochloride, the lower dose tested. It is possible that this effect may have occurred at even lower dosages and that, therefore, the CS values calculated for these studies may not be sufficiently conservative. The CS of 4.0 associated with reduced rate of body weight gain in rats (NCI, 1979) was selected as most stringently representing the toxicity of p-phenylenediamine, and is presented in Table 9-2.

TABLE 9-2

p-Phenylenediamines

Minimum Effective Dose and Reportable Quantity (RQ)

Route:	oral
Dose*:	224.0 mg/day
Effect:	slightly reduced rate of body weight gain
Reference:	NCI, 1979 (rat study)
RV_d:	2.0
RV_e:	2
Composite Score:	4.0
RQ:	5000

*Equivalent Human Dose

Phenylenediamine.

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APPENDIX A

LITERATURE SEARCHED

This profile is based on data identified by computerized literature searches of:

CA SEARCH (Files 308, 309, 310, 311, 320)
TOXLINE
RTECS
OHM TADS
STORET
SRC Environmental Fate Data Bases
SANSS
AQUIRE
TSCAPP

These searches were conducted in July, 1984. In addition, hand searches were made of Chemical Abstracts (Collective Indices 6 and 7th), and the following secondary sources were reviewed:

ACGIH (American Conference of Governmental Industrial Hygienists). 1980. Documentation of the Threshold Limit Values, 4th ed. (Includes Supplemental Documentation, 1981, 1982, 1983). Cincinnati, OH. 486 p.

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Grayson, M. and D. Eckroth, Ed. 1978-1983. Kirk-Othmer Encyclopedia of Chemical Technology, 3rd ed. John Wiley and Sons, NY. 23 Volumes.

Hamilton, A. and H.L. Hardy. 1974. Industrial Toxicology, 3rd ed. Publishing Sciences Group, Inc., MA. 575 p.

ITII (International Technical Information Institute). 1982. Toxic and Hazardous Industrial Chemicals Safety Manual for Handling and Disposal with Toxicity and Hazard Data. ITII, Tokyo, Japan. 700 p.

NTP (National Toxicology Program). 1983. Carcinogenesis Testing Program. Chemicals on Standard Protocol. Management Status.

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In addition, the following documents were obtained and reviewed:

U.S. EPA. 1978. Chemical Hazard Information Profile: Phenylenediamines. Prepared by Mitre Corporation for the U.S. EPA, Washington, DC.

U.S. EPA. 1984. Draft Technical Support Document: Phenylenediamines. Prepared by Dynamac Corporation under Contract No. 68-01-6530 for the U.S. EPA, Office of Pesticides and Toxic Substances, Washington, DC.

APPENDIX B

Cancer Data Sheet for Derivation of q_1^*

Compound: o-phenylenediamine dihydrochloride

Reference: Weisburger et al., 1978

Species, Strain, Sex: rat, Charles River, male

Body Weight: 0.35 kg (assumed)

Length of Exposure (l_e) = 548 days

Length of Experiment (L_e) = 730 days

Lifespan of Animal (L) = 730 days

Tumor Site and Type: liver, unspecified

Route, Vehicle: oral, diet

Experimental Doses or Exposures (ppm o-phenylenediamine • 2 HCl)	Transformed Dose mg/kg/day as o-phenylenediamine	Incidence No. Responding/ No. Tested (or Examined)
0	0	0/16
1000	22.4	0/14
2000	44.8	5/16

Unadjusted q_1^* from study = 8.009×10^{-3} $(mg/kg/day)^{-1}$

Human q_1^* = 4.68×10^{-2} $(mg/kg/day)^{-1}$

APPENDIX C

Cancer Data Sheet for Derivation of q_1^*

Compound: o-phenylenediamine dihydrochloride

Reference: Weisburger et al., 1978

Species, Strain, Sex: mouse, HaM/ICR, male

Body Weight: 0.030 kg (assumed)

Length of Exposure (l_e) = 548 days

Length of Experiment (L_e) = 639 days

Lifespan of Animal (L) = 730 days

Tumor Site and Type: liver, unspecified

Route, Vehicle: oral, diet

Experimental Doses or Exposures (ppm o-phenylenediamine • 2 HCl)	Transformed Dose mg/kg/day as o-phenylenediamine	Incidence No. Responding/ No. Tested (or Examined)
0	0	0/14
6,889	459.0	5/17
13,778	917.4	3/14

Unadjusted q_1^* from study = 7.576×10^{-4} $(mg/kg/day)^{-1}$

Human q_1^* = 1.50×10^{-2} $(mg/kg/day)^{-1}$

APPENDIX D

Cancer Data Sheet for Derivation of q_1^*

Compound: o-phenylenediamine dihydrochloride

Reference: Weisburger et al., 1978

Species, Strain, Sex: mouse, HaM/ICR, female

Body Weight: 0.03 kg (assumed)

Length of Exposure (l_e) = 548 days

Length of Experiment (L_e) = 639 days

Lifespan of Animal (L) = 730 days

Tumor Site and Type: liver, unspecified

Route, Vehicle: oral, diet

Experimental Doses or Exposures (ppm o-phenylenediamine • 2 HCl)	Transformed Dose mg/kg/day as o-phenylenediamine	Incidence No. Responding/ No. Tested (or Examined)
0	0	1/15
6,889	459.0	6/18
13,778	917.4	6/15

Unadjusted q_1^* from study = 9.742×10^{-4} $(mg/kg/day)^{-1}$

Human q_1^* = 1.93×10^{-3} $(mg/kg/day)^{-1}$